



Mediterranean trees coping with severe drought: Avoidance might not be safe



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ABSTRACT

Plants coexisting in Mediterranean regions differ widely in their sensitivity to drought and in their ability to maintain carbon assimilation and hydraulic function ranging from avoidance to tolerance of stressful periods. We studied the response of three coexisting Mediterranean tree species (*Quercus ilex* L. spp. *Ballota* (Desf.) Samp., *Quercus faginea* Lam. and *Pinus nigra* ssp. *Salzmannii* J.F. Arnold) under natural conditions for three years to test their functional strategies in dealing with extremely dry years. Despite *P. nigra* following a drought-avoiding strategy, it was unable to effectively avoid the hampering effects of a severe drought in the driest year, and experienced significant drops in the efficiency of the hydraulic system, in gas exchange at the leaf level and in tree water use estimated from daily sap flow. In contrast, the two *Quercus* species showed a more drought-tolerant strategy by maintaining a more profligate water use at the leaf and tree level, with *Q. ilex* having a larger tolerance threshold to severe drought. The main decrease in carbon assimilation was due to stomatal closure, with a minor effect of photochemistry damage or down regulation in the three species. In the case of the two oaks, seasonal osmoregulation was inferred from changes in the osmotic potential at full turgor (π_0), bringing about the lowest leaf osmotic potentials at turgor lost (π_{tip}) at late summer in both species, and maintenance of leaf turgor under the lowest midday leaf water potential (Ψ_{mid}). Seasonal changes in osmotic potentials were observed in the three years, more during the driest year. Plasticity in modulating the tolerance to leaf dehydration as the season advances, especially in response to the intensity of inter-annual summer droughts was higher for the evergreen *Q. ilex* than in the semi-deciduous *Q. faginea*. In spite of the intensity of the drought and the associated stress experienced by the three forest tree species, especially by *P. nigra*, mortality or severe defoliation were not observed after the extreme dry year. This reveals a large operational range of coping with water stress by the three species regardless of the hydric strategy, which is crucial for survival under the increasing drought expected in the most likely climate change scenarios for the region.

1. Introduction

Coexisting tree species in Mediterranean regions differ widely in their sensitivity to drought (Martínez-Vilalta et al., 2003; Quero et al., 2011), and in their ability to maintain carbon assimilation controlling water loss and keeping a functional hydraulic system during the most stressful periods (Nardini and Tyree, 1999; Trifilò et al., 2014). These species have developed several adaptations along a range between two opposing strategies: avoidance vs. tolerance of drought stress (Franks et al., 2007; Tardieu and Simonneau, 1998). However, differentiation

between both strategies is not easy particularly among closely related species, and classification as drought-avoiding or drought-tolerant species is not always clear (Franks et al., 2007; Klein, 2014; Attia et al., 2015). In general, drought-avoiding species have an effective and early stomatal control in response to soil water scarcity (Rambal, 1992; Attia et al., 2015), with a limited carbon assimilation (Galiano et al., 2011). This strategy can be effective during a brief period of water deprivation, which can even increase plants' tolerance to subsequent stresses, increasing its productivity in the long term. However, this potential improvement does not occur under severe droughts (Bruce et al., 2007;

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Fleta-Soriano and Munné-Bosch, 2016). Under chronic or extreme water stress, woody species can show evidence of ‘cavitation fatigue’ (Hacke and Sperry, 2001), which increases their vulnerability to successive phenomena of cavitation and therefore, to deterioration of the hydraulic system in the long term (Savi et al., 2015). Moreover, decreases in carbon fixation under severe droughts might not only be due to stomatal closure, but also to impairment of the photosynthetic system itself. Damage suffered to PSII causes photo-inhibition, decreases in the effective quantum yield, or even the triggering of photorespiration (Horton et al., 1996). These species also have other functional adaptations such as increasing root/shoot ratio (Peña-Rojas et al., 2005), a deeper root system (David et al., 2007), and even a deciduous leaf habit under drought conditions (Bréda et al., 2006; Peguero-Pina et al., 2015). Drought-tolerant species can maintain a positive carbon balance over more extended periods, avoiding photosystem damage under drought even at low leaf and xylem water potentials (Rambal et al., 2003), but lead to potentially higher risks of hydraulic failure (Choat et al., 2012). These species have some other functional adaptations, such as a high daily and seasonal osmoregulation capacity (Meinzer et al., 2014), which increases tolerance to maintain function under higher degrees of dehydration (Bartlett et al., 2014) and sclerophyllous leaves (Salleo and Lo Gullo, 1990), which minimize passive water losses under extreme drought conditions. Independently of the species’ strategy, once species thresholds of resistance are exceeded, irreversible damages and even death can occur in forest trees (Allen et al., 2010). In fact, decline and even mortality of Mediterranean forest tree species is already occurring as a consequence of severe drought events (Klein, 2015; McDowell, 2011). Since more frequent extreme droughts have been predicted under climate change in the Mediterranean region for the future (Della-Marta et al., 2007; IPCC, 2014), any species could be operating closer to its drought-tolerance limits.

Switching from drought-tolerant to drought-avoiding strategy could be a common process for most species, according to levels of water stress endured at specific times of the growing season (Rogiers et al., 2011). Species also differ regarding their specific plastic responses of different functional traits related to water availability (Nolan et al., 2017), so framing species within particular strategies should take into account the complex assemblage of different traits and their respective plasticity (Gimeno et al., 2009; Martínez-Vilalta et al., 2003; Martínez-Vilalta and García-Forner, 2016; Volaire, 2018).

We have explored drought tolerance vs. avoidance in two oaks and one pine species coexisting in vast areas of continental Mediterranean climate in the interior of the Iberian Peninsula. *Quercus ilex* L. is a typical evergreen sclerophyllous tree from the Mediterranean Basin with a distribution spanning over a large geographical and altitudinal range from high altitudes to near-coastal sites (Niemets, 2015). A large number of studies in these climates have focused on this species due to its high tolerance to drought (Misson et al., 2010; Moreno and Cubera, 2008; Niinemets, 2015; Peñuelas et al., 1998). Long and intense drought events could bring about *Q. ilex* to exceed its safety margins and show symptoms of hydraulic failure (Urli et al., 2013), which could also happen with the coexisting winter deciduous oak *Quercus faginea*. *Q. faginea* also has a relatively high tolerance to summer drought, given this is not very intense and does not last very long (Esteso-Martínez et al., 2006). Its ability to adapt to semiarid climates is aided by reducing its leaf area under extreme droughts, but having high stomatal and mesophyll conductance when soil moisture availability is high allows for fast spring growths (Mediavilla et al., 2002; Peguero-Pina et al., 2016). *Pinus nigra* coexists with both *Quercus* species in some Mediterranean forests and shows high vulnerability to drought (Forner et al., 2014, 2018). *P. nigra* closes stomata early under drought to avoid significant leaf water potential decreases and hydraulic cavitation, compromising its carbon assimilation and therefore its growth (Forner et al., 2014; Martín-Benito et al., 2008).

We determined differences in response to severe drought of adult

trees under real field conditions of these three coexisting Mediterranean tree species. Functional performance of selected individuals was analyzed during three years of contrasting drought intensity, according to the extent of the soil dry-drown season and the duration of the effective seasonal drought (Forner et al., 2014). Moreover, we assessed whether decrease in net photosynthetic rate under drought conditions was primarily due to stomatal closure or to damage to the leaf biochemistry. We quantified osmoregulation as a drought-tolerant mechanism, and evaluated together with changes in the bulk leaf modulus of elasticity in the two *Quercus* species. We hypothesized that under acute dry seasons, drought-tolerant species maintain carbon assimilation and the functionality of their hydraulic system by an efficient strategy that combines mechanisms of avoidance and tolerance to drought. Thus, it was expected that the two *Quercus* species maintain a more profligate use of water, but in return would be able to maintain higher leaf carbon assimilation rates than *Pinus nigra* in response to drought, especially during the driest years.

2. Materials and methods

2.1. Study site and meteorological data

The study was carried out in a stand at the Armallones site (40°46'37"N, 2°19'42"W, 1079 m a.s.l.), located in the Alto Tajo Natural Park, Guadalajara (central Spain). The climate is continental Mediterranean with hot and dry summers and cold winters. The main woody species have similar basal area, and are *Quercus faginea* (38.7%), *Pinus nigra* (35.1%) and *Quercus ilex* (25.8%). For more details of the study site see Granda et al. (2013) and Forner et al. (2014).

We randomly-selected five healthy and fully-sun exposed representative dominant trees with similar DBH (diameter at breast height, 1.3 m) of Holm oak, *Q. ilex* subsp. *ballota*, Portuguese oak, *Q. faginea* and black pine, *P. nigra* subsp. *salzmanii* (total $n = 15$).

Meteorological conditions were recorded continuously from 2011 to 2013 at the study site using a HOBO® (Onset Computer Co., Bourne, MA, USA) weather station, which recorded every 30 min the rain, temperature, relative air humidity, solar radiation and wind speed (see Appendix B.1 for more information). Air vapor pressure deficit (VPD, kPa) was calculated according to Campbell and Norman (1998), from the atmospheric pressure, the air temperature and the air relative humidity. The relative extractable water in the soil (REW) was calculated according to Granier et al. (1999). REW below the threshold of 0.4 indicates water stress reached levels in which water is not available to tree roots, limiting transpiration, and causing high stomatal closure for most temperate forest tree species (Granier et al., 1999).

During the study period (2011–2013) daily temperature and VPD varied between 27.2–18.5 °C and 2.9–1.5 kPa in early summer (ESu), and 23.5–19.1 °C and 2.5–1.2 kPa in late summer (LSu), respectively. The year 2012 was the driest year: it had the highest temperature and VPD and the lowest REW in both, early and late summer (Fig. 1, and see Table B.1 in supplementary material). Moreover, ESu of 2012 was even drier than the LSu of the three studied years. There was no precipitation on the days prior to the days in which measurements were made. (For more information about drought conditions in the study period see Forner et al. (2018)).

2.2. Water use and water status at the tree level

Water stress endured by trees was assessed from predawn leaf water potential (Ψ_{pd} , MPa) measured in two leaves per tree for each species (five trees per species) at twice through the growing season in all three years of the study (ESu and LSu; 2011–2013). Excised leaves were collected before predawn and enclosed into sealable plastic bags and kept refrigerated in the dark until the measurement was done half an hour after collecting samples (Pérez-Harguindeguy et al., 2013). Leaf water potential was measured again in the afternoon (from 1 to 3 p.m.)

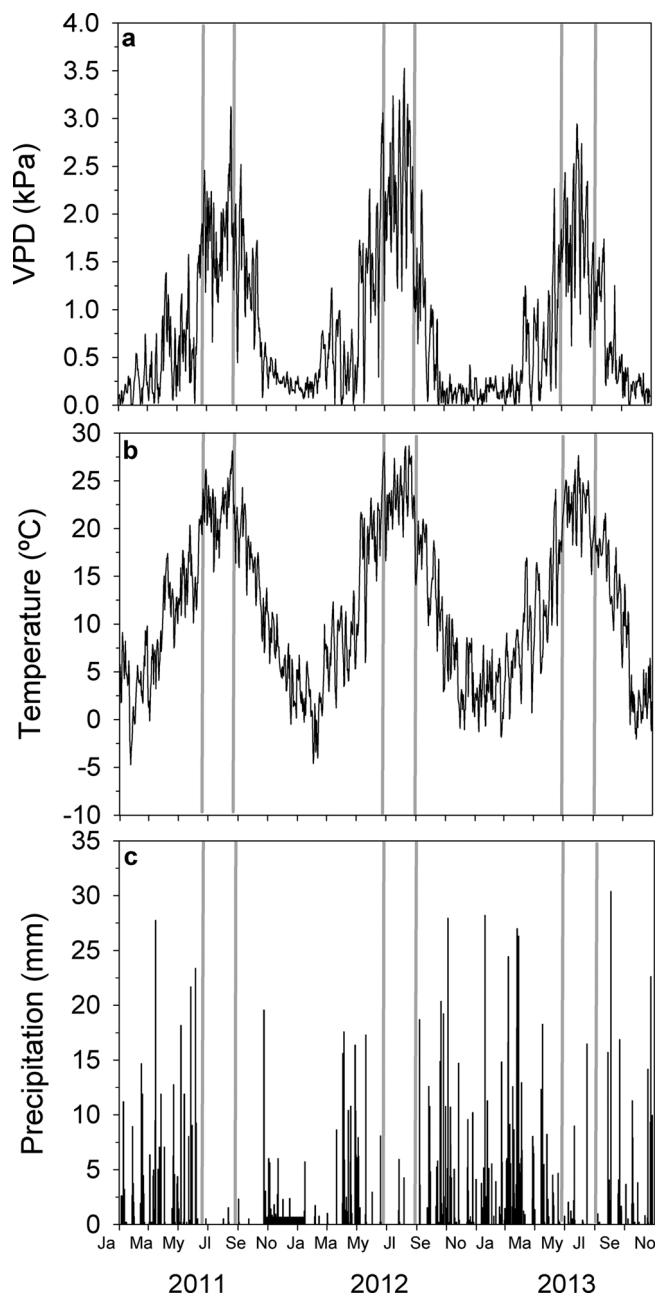


Fig. 1. Mean monthly vapor pressure deficit (VPD, kPa) (a) and temperature (T, °C) (b), and total monthly precipitation (P, mm) (c) for each study year (2011–2013). Grey lines divide the two sampling seasons, early and late summer.

in transpiring (Ψ_{leaf} , MPa) and non-transpiring leaves as surrogate of xylem water potential (Ψ_{xyl} , MPa). All leaf water potential measurements were performed using a Scholander pressure chamber (PMS 1000, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (see Appendix B.2 for more information).

Water use at tree level was monitored using sap flow sensors EMS 51 (Environmental Measuring Systems, Brno, Czech Republic) over a week of measurements during the two sampling seasons, ESu and LSu, all three years (2011–2013). Daily sap flow per basal area (Q_v , $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$) was measured for three days, including the day of gas exchange measurements for each species at the two sampling seasons (ESu and LSu) and for the three years of study (2011–2013). We also calculated the mean of total daily sap flow per basal area (Q_{v7} , $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$) measured for a full week in the same seasons and years,

and the percentage reduction of the total daily sap flow per basal area from the maximum recorded during the study period (RM, %).

Three hydraulic conductances were calculated by analogy to the Ohm's law (van den Honert, 1948) for the five trees per species and for ESu and LSu in each of the study years (2011–2013): leaf-specific apparent hydraulic conductance in the soil-plant-atmosphere continuum (K_h , $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$), the branching system apparent hydraulic conductance (K_b , $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) and petiole-leaf lamina apparent hydraulic conductance (K_{pl} , $\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) (see Appendix B.2 for information).

2.3. Leaf functional traits

Leaf functional traits were measured in ESu and LSu in the three study years. We selected two current-year shoots per tree with fully expanded and sun leaves without symptoms of herbivore damage. Specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$) was estimated from fresh leaves without their petioles scanned and oven-dried at 65 °C for 48 h. Leaf area was estimated with the software ImageJ from scanned leaves (Rasband, 2009). Elemental leaf carbon and leaf nitrogen content (C_m and N_m , respectively; %) were obtained from the same leaves (see Appendix A.3 for more information). We also calculated the ratio between C_m and N_m (C_m/N_m).

Gas exchange and chlorophyll fluorescence measurements were performed on two healthy, fully developed leaves from the same trees of each species selected for all measurements ($n = 15$) using a Li-Cor 6400 XT (Li-Cor, Inc., NE) gas exchange system with an integrated fluorescence chamber head (Chamber Li-6400-40, Li-Cor, Inc., NE) during ESu and LSu of each of the study years (2011–2013). Measurements were carried out in the morning (M, from 10:30 a.m. to 1 p.m.) and the afternoon (A, from 1 to 3 p.m.). Net photosynthetic rate on a leaf area basis (A_{area} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance of water vapor (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$ of H_2O), effective quantum yield ($\Delta F'/F_m'$), photochemical quenching (qP) and non-photochemical quenching (qN) were measured (see Appendix B.3 for more information). Photosynthetic nitrogen-use efficiency (PNUE, $\mu\text{mol mg}^{-1} \text{s}^{-1}$ of CO_2 per N) was calculated as the ratio between A_{area} and leaf nitrogen content per area (N_a), which in turn was calculated dividing N_m by SLA.

2.4. Pressure-volume curves

Pressure-volume curves were constructed from leaves collected on the same shoots where gas exchange and water stress of trees were recorded in the three years. Parameters from P–V curves were estimated from leaves of the two oaks to better assess differences in the leaf water relations between both species as they are closer from phylogeny and water use strategy. Pressure-volume curves were constructed according to the previously well-established protocol for the free-transpiration method (Aranda et al., 1996; Corcuera et al., 2002; Dreyer et al., 1990) (see Appendix A.4 for more information). From the pressure-volume curves we estimated the following: the relative water content at the turgor lost point (RWC_{tlp} , %), relative apoplastic water content (RWC_a , %), modulus of elasticity (ϵ , MPa), osmotic potential at full turgor (π_0 , MPa), osmotic potential at the turgor lost point (π_{tlp} , MPa) and ratio of leaf dry weight to leaf turgid weight (DW/TW).

2.5. Statistical analysis

Linear models were used to test differences in meteorological variables (temperature, precipitation and vapor pressure deficit) among years. Linear mixed-effects models (LMMs) were used to assess the effect of year, season (ESu and LSu) and their interaction (fixed factors) on variables related to leaf traits (SLA, C_m , N_m , C_m/N_m , PNUE), gas exchange and chlorophyll fluorescence traits (A_{area} , g_s , $\Delta F'/F_m'$, qP , qN), tree water use (Q_v , Q_{v7} , RM), water status (Ψ_{pd} , Ψ_{leaf} , Ψ_{xyl} , K_h , K_b),

K_{pI}) and water parameters from pressure-volume curves (RWC_{tp} , RWC_{a} , ϵ , π_0 , π_{tp}) (Zuur et al., 2007). Tree identity was considered as random in the LMMs. We performed LMMs by species to analyze how year, season and meteorological conditions could affect traits differently depending on species. Models were fitted based on a restricted maximum likelihood method using the nlme package from R statistical software (Pinheiro et al., 2007). The Kruskal-Wallis test was used when data were not normal.

Relationships between functional traits were established by fitting linear and non-linear models according to species and season. The instantaneous water-use efficiency (iWUE) was calculated from the slope of the regression between net photosynthetic rate and stomatal conductance to water vapor. The relationship between soil available water and available water for trees was assessed by fitting regression curves between relative extractable water and predawn water potential by species. Regressions were performed using SigmaPlot version 11, from Systat Software, Inc., San Jose California USA. Adjusted R-squared (R^2) was indicated in all cases.

Spearman's rank correlation analysis was used to establish relationships among all functional traits. Results were shown using

correlograms which were performed using the package corrrplot from R statistical software. All analyses were carried out using the R statistical software (version R3.2.3; R Core Team, 2015, Vienna), if not otherwise indicated.

3. Results

3.1. Water status and water use: differences among species and years

Water stress endured by trees of the three species, as assessed from predawn leaf water potential (Ψ_{pd}), was higher in late summer (LSu) than in early summer (ESu). From most of the dates *P. nigra* had lower Ψ_{pd} than the two *Quercus* species, which had similar Ψ_{pd} (Fig. 2a, b). The decrease in Ψ_{pd} between ESu and LSu was the highest in 2012 in the two *Quercus* species (*Q. ilex*: -2.09 MPa and *Q. faginea*: -0.97 MPa). Ψ_{pd} in the three species depended on the interaction between season and year (Table B.2). Ψ_{pd} was more sensitive to relative extractable water (REW) in *P. nigra* than in the two *Quercus* species (Fig. B.1). At the threshold of $\text{REW} = 0.4$, *P. nigra* showed the lowest Ψ_{pd} (-1.12 MPa ; *Q. ilex*: -0.67 MPa and *Q. faginea*: -0.53 MPa). At $\text{REW} = 0.1$, the two *Quercus* species

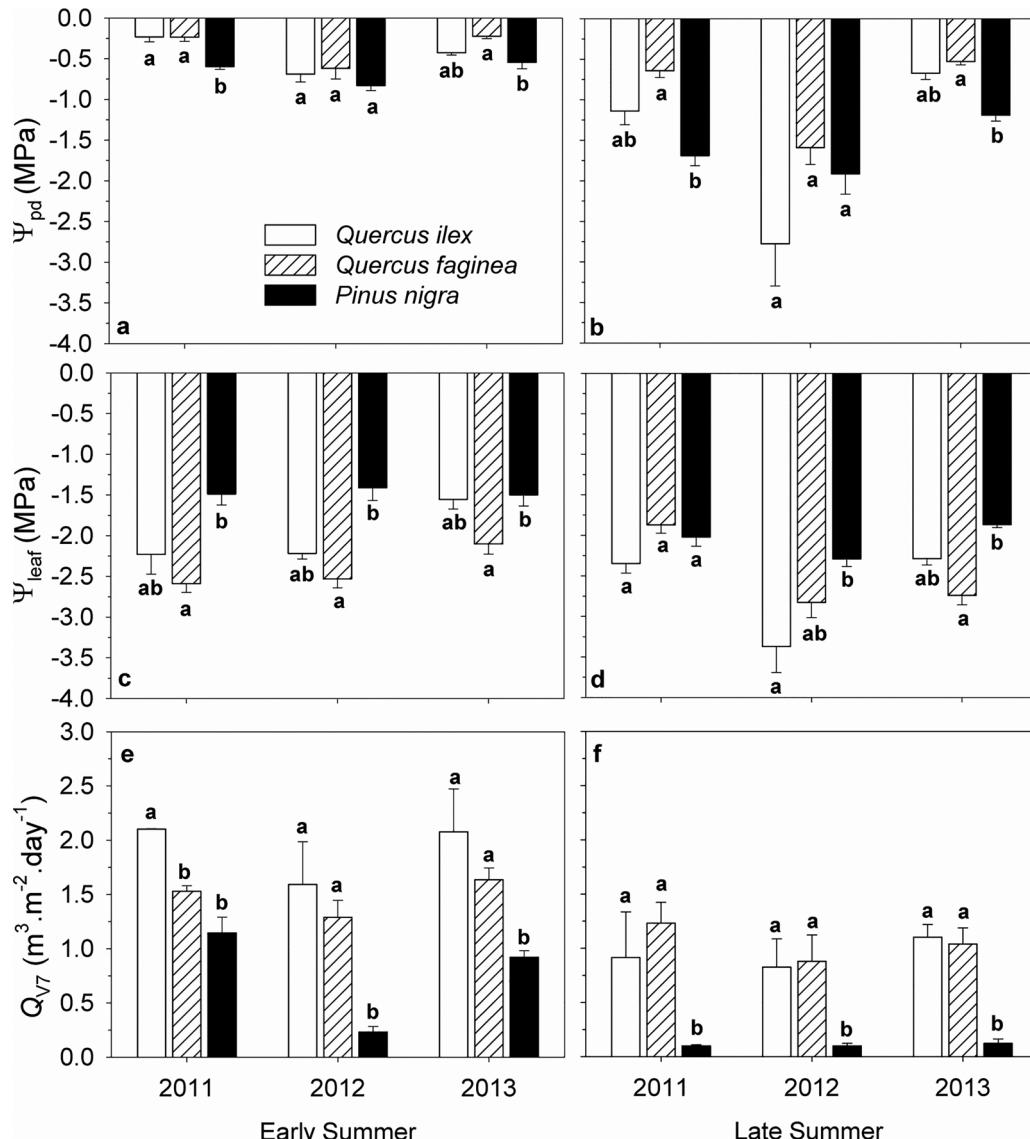


Fig. 2. Mean \pm SE of predawn leaf water potential (Ψ_{pd} , MPa; a, b), afternoon leaf water potential (Ψ_{leaf} , MPa; c, d) and total daily sap flow per basal area (Q_{v7} , $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$; e, f) measured for a week at the two sampling seasons, early (a, c, e) and late summer (b, d, f) for each species and year (2011–2013). Small letters indicate significant differences between species in each sampling season and year at $P < 0.05$.

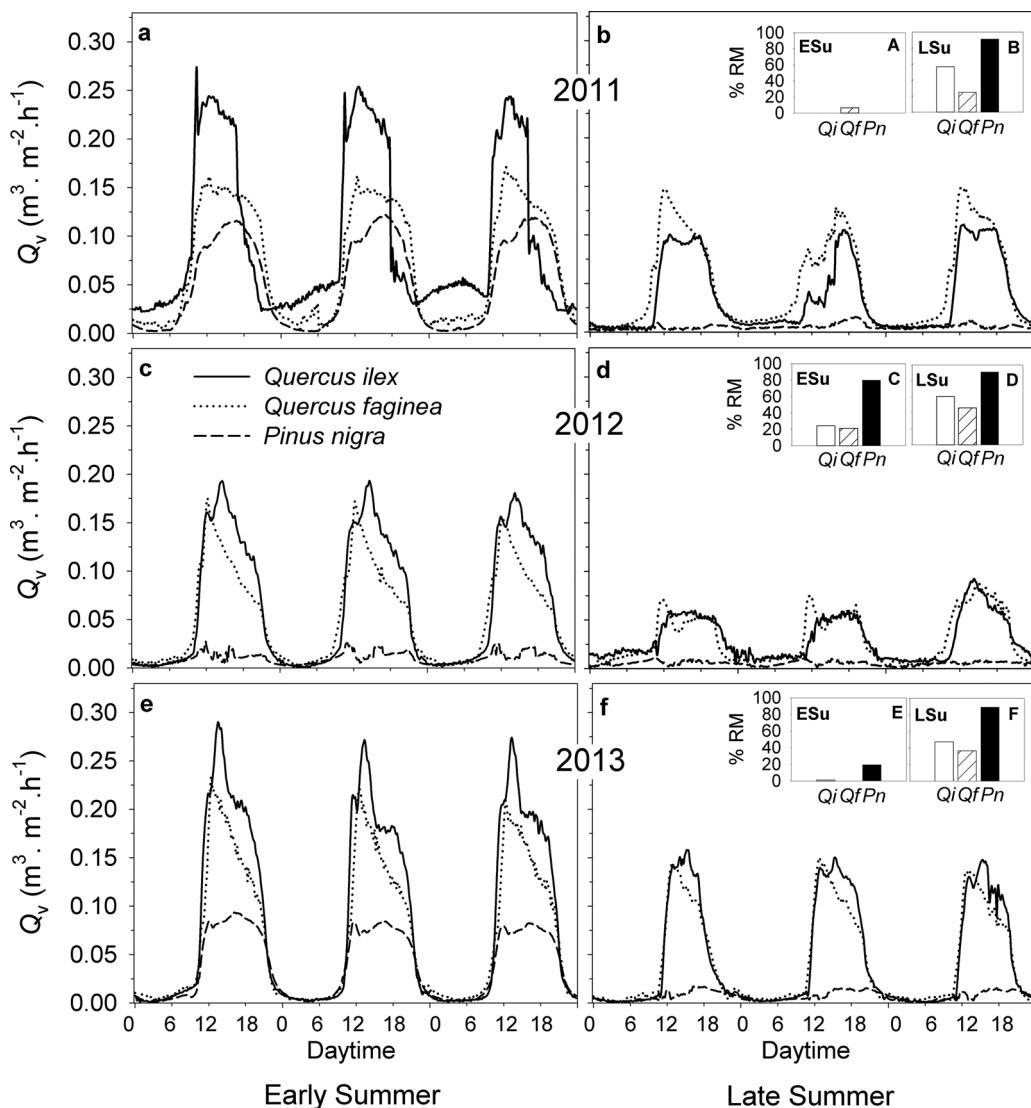


Fig. 3. Daily sap flow per basal area (Q_v , $\text{m}^3 \text{m}^{-2} \text{h}^{-1}$) measured at the two sampling seasons, early (a, c, e) and late summer (b, d, f) for each species and year (2011: a, b; 2012: c, d; 2013: e, f). Small inset figures show the percentage reduction of the total daily sap flow per basal area from the maximum, measured for the week of sampling at early (ESu; A, C, E) and late summer (LSu; B, D, F) seasons for each species (Qi: *Quercus ilex*; Qf: *Quercus faginea*; Pn: *Pinus nigra*) and year (2011: A, B; 2012: C, D; 2013: E, F).

significantly decreased their Ψ_{pd} and reached similar (*Q. faginea*) and lower (*Q. ilex*) values compared to *P. nigra*.

Afternoon leaf water potential (Ψ_{leaf}) was higher in *P. nigra* (Fig. 2c, d). *P. nigra* was the only species without significant differences in Ψ_{leaf} between years apart from the intensity of summer drought (Table B.2).

Daily and weekly water use values estimated from daily sap flow per basal area (Q_v and Q_{v7} respectively) were lower in *P. nigra* than the two *Quercus* species in both seasons and for all study years (Figs. 2e, f; 3). *Q. ilex* had higher water use than *Q. faginea* in ESu, but slightly lower in LSu (Fig. 2e, f). Differences between both *Quercus* species were higher in 2011. In the driest year, 2012, all species had low water use, even in ESu *P. nigra* already decreased Q_v to similar levels as in LSu. In the three years, the decrease in water consumption from early to late summer was more noticeable in *P. nigra*, with a diminution over the three summers of more than 80% from maximum measured in ESu of 2011 (Fig. 3b–d, f, and insets A–F). Moreover, in ESu of 2012, *Q. faginea* produced an earlier and faster daily decrease in Q_v in the afternoon than *Q. ilex* (Fig. 3c). Water consumption, from analysis of Q_v , depended only on the season in *Q. ilex*, but also on the year for *Q. faginea*, and in the case of *P. nigra* both factors and their interaction influenced the water use of the species (Table B.2).

In general, all three species had lower leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h) in the driest year, 2012, in both ESu and LSu (Fig. 4). Reductions in K_h between 2011 and the driest year, 2012, were higher in *Q. faginea* in both sampling seasons, but with similar percentage decrease in *P. nigra* in LSu (ESu: 69% *Q. ilex*, 77% *Q. faginea*, 57% *P. nigra*; LSu: –14% *Q. ilex* (increase), 79% *Q. faginea*, 77% *P. nigra*). The year and its interaction with season was an important factor determining K_h in the two *Quercus* species, while in *P. nigra* K_h only depended on year (Table B.2).

In LSu, g_s was not correlated with hydraulic conductances in *Q. ilex*. However, *Q. faginea* and *P. nigra* showed a significant positive correlation between g_s and the different apparent hydraulic conductances in the continuum soil-plant-atmosphere (K_h , K_b and K_{pl} ; Fig. 5a–c).

3.2. Species and year differences in leaf functional traits

In general, *Q. faginea* had the highest specific leaf area (SLA), elemental leaf nitrogen content (N_m) and photosynthetic nitrogen-use efficiency (PNUE), and *P. nigra* had the lowest values of these traits (Table 1). Contrary, *Q. faginea* had the lowest ratio between elemental leaf carbon content (C_m) and N_m (C_m/N_m), and *P. nigra* the highest

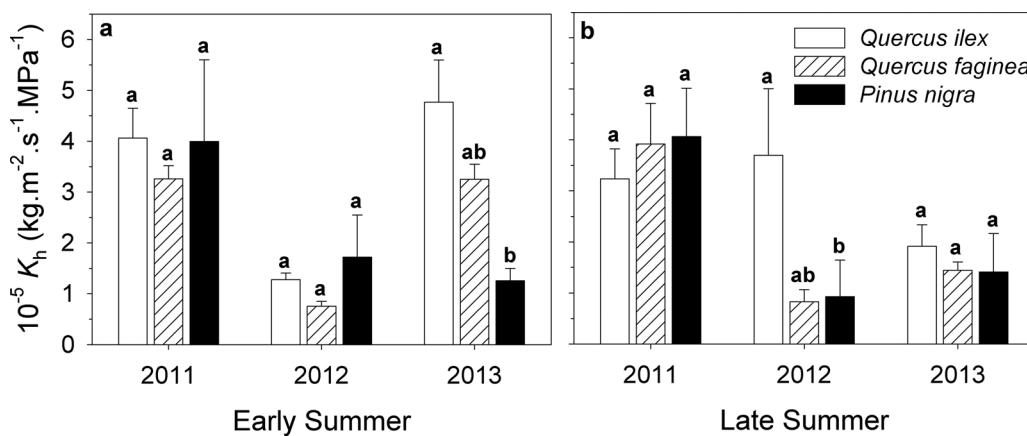


Fig. 4. Mean \pm SE of leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h , $10^{-5} \text{ kg m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$; a, b) calculated at the two sampling seasons, early (a) and late summer (b), for each study species and study year (2011–2013). Small letters indicate significant differences between species in each sampling season and year at $P < 0.05$.

values. Season had an effect on PNUE in *Q. ilex*, N_m in *Q. faginea* and SLA, N_m and PNUE in *P. nigra* (Table B.2), with higher values measured in ESu. The ratio C_m/N_m was significantly higher in LSu in *Q. faginea* and *P. nigra*. SLA and PNUE depended on year in all three species, and the highest SLA and lowest PNUE were measured in the driest year (2012) (Table 1, S2). In LSu of 2012, *P. nigra* reduced PNUE in a 96% and 95% compared with the same season of 2011 and 2013, respectively. N_m only varied significantly between years in *Q. ilex*, being lower in 2012.

Regarding correlations among leaf traits, leaf water potential and apparent hydraulic conductance, *Q. ilex* only showed correlations between PNUE and Ψ_{pd} , Ψ_{xyl} , K_b and K_{pl} (Fig. 5a). In *Q. faginea* SLA was correlated with Ψ_{pd} and Ψ_{xyl} and PNUE with Ψ_{leaf} , Ψ_{xyl} , K_h and K_b (Fig. 5b). *P. nigra* was the species with most correlations among these variables (Fig. 5c).

Net photosynthetic rate on a leaf area basis (A_{area}) and stomatal conductance to water vapor (g_s) followed similar patterns. The highest values were measured in *Q. faginea* in the morning of ESu, and the lowest values in *P. nigra*. The lowest leaf gas exchange values were recorded in 2012 for all species (Fig. 6a–d). Differences in g_s measured in ESu of 2011 and of 2012 were higher than the observed for A_{area} in all species. However, in the driest conditions (LSu of 2012), A_{area} decreased (mean:

93%) more than g_s (mean: 81%) in *P. nigra*. Reductions in g_s between morning and afternoon were high in all species ($> 91\%$). Both morning and afternoon measurements of A_{area} and g_s depended on the year and season (Table B.3). A_{area} and g_s were significantly correlated in *Quercus* species but not in *P. nigra*, and in particular in the summer of 2012, when both variables were partially decoupled (Figs. 5a–c; B.2). Regressions between A_{area} and g_s measured in morning were significant for all species, with stronger relations in LSu (Fig. B.2). The slope of the regression can be related with the intrinsic water-use efficiency (iWUE), which increased for all species from ESu to LSu. In fact, *P. nigra* was the species with the largest increase of iWUE through the summer. Moreover, in ESu A_{area} and g_s , and therefore iWUE, of *P. nigra* did not depend on Ψ_{pd} , but they did in LSu (Fig. 7a–d). The same trend was observed in *Q. faginea* between g_s and Ψ_{pd} (Fig. 7c, d). In the driest conditions (LSu), and at a same Ψ_{pd} *P. nigra* had the lowest A_{area} and g_s , while both *Quercus* species had similar values of A_{area} and g_s between -1 and -3 MPa of Ψ_{pd} . Higher water potentials (> -1 MPa) allowed higher A_{area} and g_s in *Q. faginea* than in *Q. ilex*, while lower values of Ψ_{pd} (< -3 MPa) were only recorded in *Q. ilex*. In fact, both *Quercus* species showed significant correlations between A_{area} and g_s and Ψ_{pd} , Ψ_{leaf} and Ψ_{xyl} but these correlations were not apparent for *P. nigra* (Fig. 5a–c).

Effective quantum yield ($\Delta F'/Fm'$) was slightly higher in ESu than in

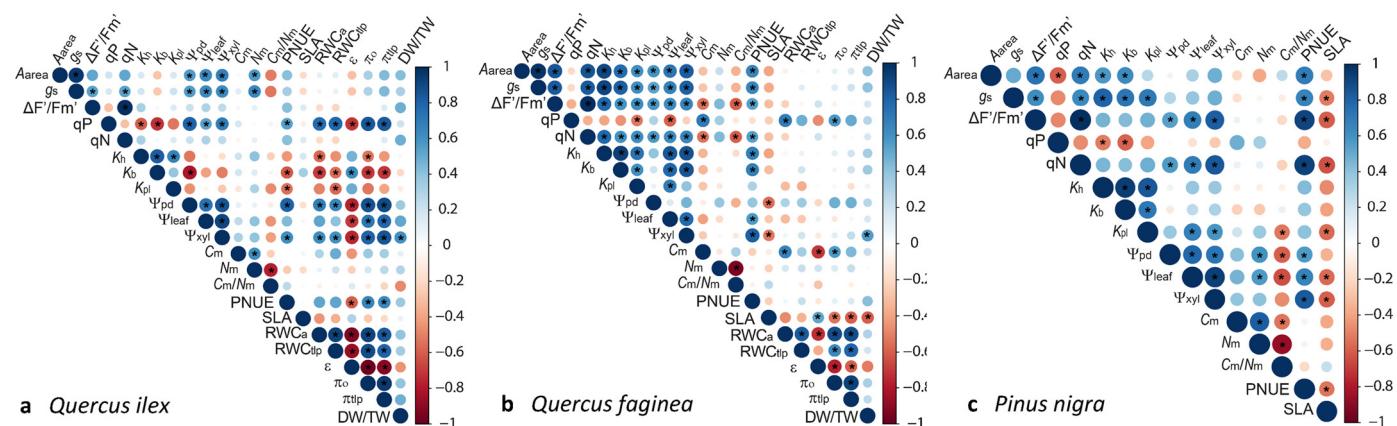


Fig. 5. Correlograms for *Quercus ilex* (a), *Quercus faginea* (b) and *Pinus nigra* (c); Spearman's correlations between the net photosynthetic rate on a leaf area basis (A_{area}), stomatal conductance of water vapour (g_s), effective quantum yield ($\Delta F'/Fm'$), photochemical quenching (qP), non-photochemical quenching (qN), leaf-specific apparent hydraulic conductance in the soil-plant-air continuum (K_h), branching system apparent hydraulic conductance (K_b), petiole-leaf lamina apparent hydraulic conductance (K_{pl}), predawn leaf water potential (Ψ_{pd}), afternoon leaf water potential (Ψ_{leaf}), afternoon leaf water potential in non-transpiring leaves (Ψ_{xyl}), elemental leaf carbon content (C_m), elemental leaf nitrogen content (N_m), ratio between C_m and N_m (C_m/N_m), photosynthetic nitrogen-use efficiency (PNUE), specific leaf area (SLA), relative apoplastic water content (RWC_a), relative water content at the turgor lost point (RWC_{dp}), modulus of elasticity (ε), osmotic potential at full turgor (π_o), osmotic potential at the turgor lost point ($\pi_{t_{dp}}$) and ratio of leaf dry weight and leaf turgid weight (DW/TW) measured during late summer and in the afternoon for the gas exchange measurements. Variables related to curves P–V were not measured in *P. nigra* (RWC_a, RWC_{dp}, ε , π_o , $\pi_{t_{dp}}$ and DW/TW). Blue and red circles indicate positive and negative correlations respectively. Size of the circle and intensity of the colour indicate the magnitude of the correlation, being higher with longer size and more intense colour. Significant correlations at $P < 0.05$ are indicated with asterisk (*) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 1

Mean \pm SE of the specific leaf area (SLA, $\text{m}^2 \text{kg}^{-1}$), elemental leaf carbon content (C_m , %), elemental leaf nitrogen content (N_m , %), ratio between C_m and N_m (C_m/N_m , unitless) and photosynthetic nitrogen-use efficiency (PNUE, $\mu\text{mol mg}^{-1} \text{s}^{-1}$) at the two sampling seasons, early and late summer (ESu and LSu respectively), for each species (Qi: *Quercus ilex*; Qf: *Quercus faginea*; Pn: *Pinus nigra*) and years (2011–2013). For each year and season *different letters* indicate significant differences between species at $P = 0.05$.

		2011		2012		2013	
		ESu	LSu	ESu	LSu	ESu	LSu
SLA ($\text{m}^2 \text{kg}^{-1}$)	Qi	3.87 \pm 0.11 ^a	4.88 \pm 0.11 ^{ab}	5.97 \pm 0.26 ^a	5.43 \pm 0.37 ^a	5.20 \pm 0.25 ^a	5.01 \pm 0.21 ^{ab}
	Qf	6.78 \pm 0.50 ^b	8.13 \pm 0.20 ^a	9.04 \pm 0.18 ^b	8.74 \pm 0.25 ^b	9.16 \pm 0.43 ^b	7.91 \pm 0.15 ^a
	Pn	4.61 \pm 0.31 ^{ab}	4.15 \pm 0.10 ^b	6.63 \pm 0.11 ^{ab}	5.12 \pm 0.10 ^a	7.46 \pm 0.23 ^{ab}	3.76 \pm 0.21 ^b
C_m (%)	Qi	46.52 \pm 0.61 ^{ab}	46.09 \pm 0.29 ^a	43.67 \pm 1.63 ^a	44.55 \pm 1.84 ^a	46.50 \pm 0.19 ^a	44.73 \pm 2.49 ^{ab}
	Qf	44.01 \pm 0.76 ^a	42.98 \pm 1.14 ^a	45.70 \pm 0.43 ^a	45.34 \pm 1.27 ^a	44.25 \pm 0.73 ^a	45.00 \pm 0.35 ^a
	Pn	50.64 \pm 1.06 ^b	42.61 \pm 4.00 ^a	48.20 \pm 1.44 ^a	44.96 \pm 1.47 ^a	43.04 \pm 3.00 ^a	48.61 \pm 0.27 ^b
N_m (%)	Qi	1.17 \pm 0.07 ^{ab}	1.32 \pm 0.13 ^a	1.06 \pm 0.01 ^a	1.04 \pm 0.04 ^{ab}	1.26 \pm 0.05 ^{ab}	1.12 \pm 0.08 ^{ab}
	Qf	1.59 \pm 0.14 ^a	1.44 \pm 0.11 ^a	1.86 \pm 0.21 ^b	1.37 \pm 0.08 ^a	1.83 \pm 0.17 ^a	1.63 \pm 0.14 ^a
	Pn	0.99 \pm 0.08 ^b	0.72 \pm 0.08 ^b	1.16 \pm 0.12 ^{ab}	0.75 \pm 0.07 ^b	1.13 \pm 0.06 ^b	0.90 \pm 0.03 ^b
C_m/N_m	Qi	40.42 \pm 2.53 ^{ab}	36.16 \pm 2.94 ^a	41.27 \pm 1.73 ^{ab}	43.08 \pm 2.02 ^{ab}	37.25 \pm 1.47 ^a	40.17 \pm 1.68 ^{ab}
	Qf	28.47 \pm 2.49 ^a	30.57 \pm 2.66 ^a	26.11 \pm 3.47 ^a	33.68 \pm 2.78 ^a	24.91 \pm 2.08 ^b	28.44 \pm 2.64 ^a
	Pn	52.27 \pm 4.42 ^b	60.19 \pm 2.69 ^b	43.31 \pm 4.60 ^b	61.60 \pm 4.73 ^b	38.08 \pm 0.87 ^a	54.53 \pm 1.87 ^b
PNUE ($\mu\text{mol mg}^{-1} \text{s}^{-1}$)	Qi	54.33 \pm 1.37 ^{ab}	39.41 \pm 4.77 ^{ab}	52.30 \pm 8.19 ^a	31.21 \pm 3.01 ^{ab}	69.94 \pm 5.80 ^{ab}	51.16 \pm 6.97 ^{ab}
	Qf	84.16 \pm 15.78 ^a	81.89 \pm 17.95 ^a	52.83 \pm 8.37 ^a	46.02 \pm 4.01 ^a	101.19 \pm 10.34 ^a	62.68 \pm 5.88 ^a
	Pn	41.20 \pm 5.56 ^b	25.72 \pm 7.62 ^b	35.27 \pm 7.99 ^a	0.95 \pm 4.02 ^b	44.34 \pm 5.76 ^b	18.04 \pm 3.58 ^b

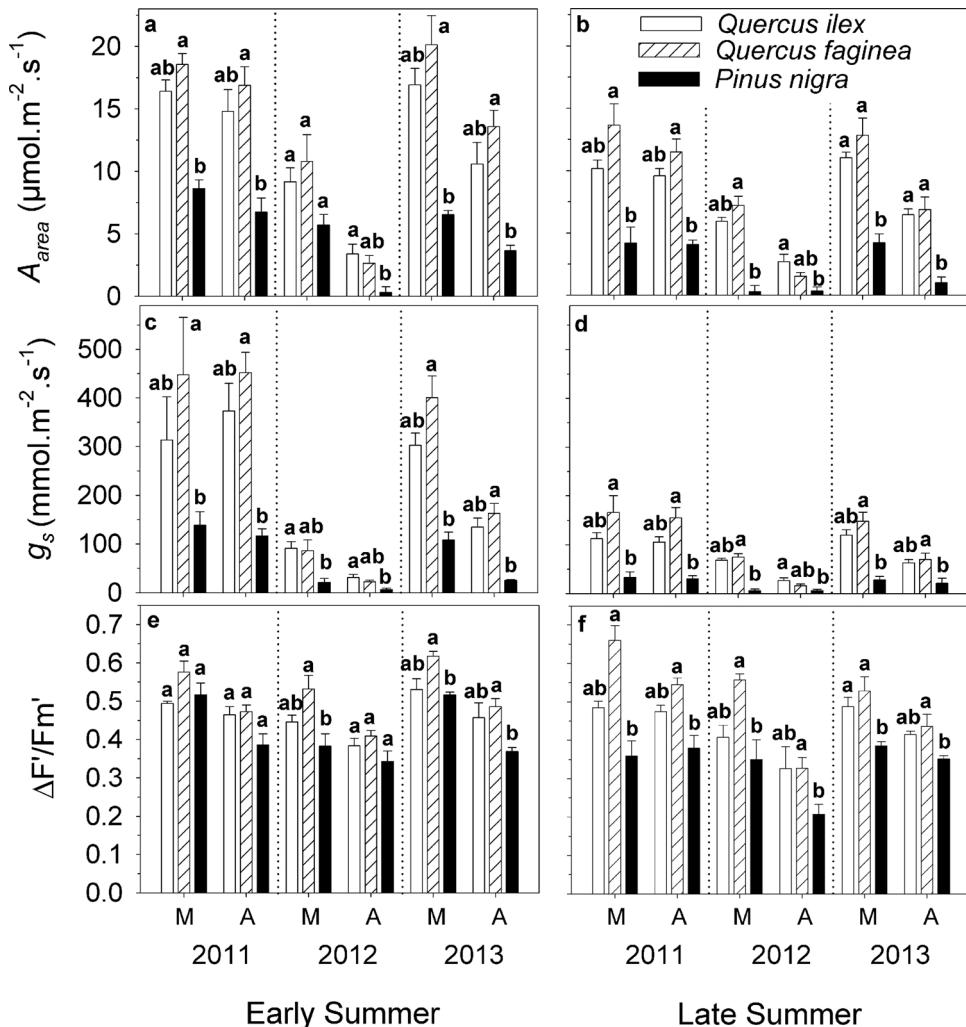


Fig. 6. Mean \pm SE of net photosynthetic rate on a leaf area basis (A_{area} , $\mu\text{mol m}^{-2} \text{s}^{-1}$; a, b), stomatal conductance of water vapor (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$; c, d) and effective quantum yield ($\Delta F'/Fm'$; e, f) measured in the morning (M) and the afternoon (A) at the two sampling seasons, early (a, c, e) and late summer (b, d, f) for each species and year (2011–2013). Dotted lines separate different years. Small letters indicate significant differences between species, each morning and afternoon, for each sampling season and year at $P < 0.05$.

LSu, in the morning than in the afternoon and in *Q. faginea* than in the other two species (Fig. 6e, f). *P. nigra* showed the lowest values of $\Delta F'/Fm'$, although in general they were not significantly different from *Q. ilex*. The driest year brought about the lowest values recorded in the three species. In *P. nigra*, $\Delta F'/Fm'$ depended on year and season, but in both *Quercus* species, seasonal $\Delta F'/Fm'$ was stable and only depended on year (Table B.3). Photochemical and non-photochemical quenching (qp and qN, respectively) did not show a clear pattern of differences between species and in response to drier conditions, although lower values of qp were observed in LSu for all species (Fig. B.3).

3.3. Pressure-volume curves in *Quercus* species

General patterns indicated that relative water content at the turgor lost point (RWC_{tlp}), osmotic potential at full turgor (π_o) and at the turgor lost point (π_{tlp}), and ratio of leaf dry weight and leaf turgid weight (DW/TW) were similar in current-year leaves of *Q. ilex* and *Q. faginea* in ESu (Table 2). In LSu, RWC_{tlp} , and the modulus of elasticity (ϵ) tend to be higher in *Q. ilex*, and also had larger DW/TW in most seasons and years. The most relevant result was that π_o and π_{tlp} decreased significantly in both species in 2012 with respect to 2011 and 2013. There were significant differences between both species in π_o and marginally non-significant in π_{tlp} only in LSu of the driest year (Table 2). The response of ϵ , π_o and π_{tlp} to Ψ_{pd} was only significant in *Q. ilex* (Fig. 8). We observed that π_{tlp} and π_o were the variables most determined by Ψ_{pd} in both species. None of water related parameters obtained from pressure-volume curves were significantly correlated with water leaf and xylem potentials or hydraulic conductances in *Q. faginea* (Fig. 5b). However, there were significant correlations among most of those parameters and Ψ_{pd} , Ψ_{xyl} , Ψ_{leaf} and K_b in *Q. ilex* (Fig. 5a).

4. Discussion

Even though *P. nigra* followed a drought-avoiding strategy, the species was not able to cope with the severe drought that occurred in 2012 without enduring substantial effects on its water use and carbon uptake capacity. The main decrease in carbon assimilation was due to stomatal closure to avoid water losses, with a minor effect in the photochemistry of the three species, although it was slightly down-regulated in the three years by late summer. In contrast, both *Quercus* species adopted more drought-tolerant strategies, but in particular *Q. ilex*, maintaining a water spender performance in especially dry days and years in agreement with previous studies (Mediavilla and Escudero, 2003; Misson et al., 2010).

All three species showed lower net photosynthetic rates during the morning and afternoon of 2012 than normally could be expected, especially in early summer for *P. nigra*. In fact, *P. nigra* showed leaf net photosynthetic rates close to photorespiration values during early summer of 2012. This could suggest the existence of another factor conditioning net photosynthetic rate in *P. nigra*, such as damage to the photosynthetic system. However, decreases in these efficiency of PSII and photochemical quenching we reported were not severe. Moreover, we found no significant increases in non-photochemical quenching. Therefore, our results confirmed the primary role of stomata as the main and early limitation to photosynthesis in these three species. Though other sources of limitation not studied at present, such as mesophyll conductance or biochemical limitations, could also have influenced leaf photosynthesis (Cano et al., 2013; reviewed in Flexas et al., 2014).

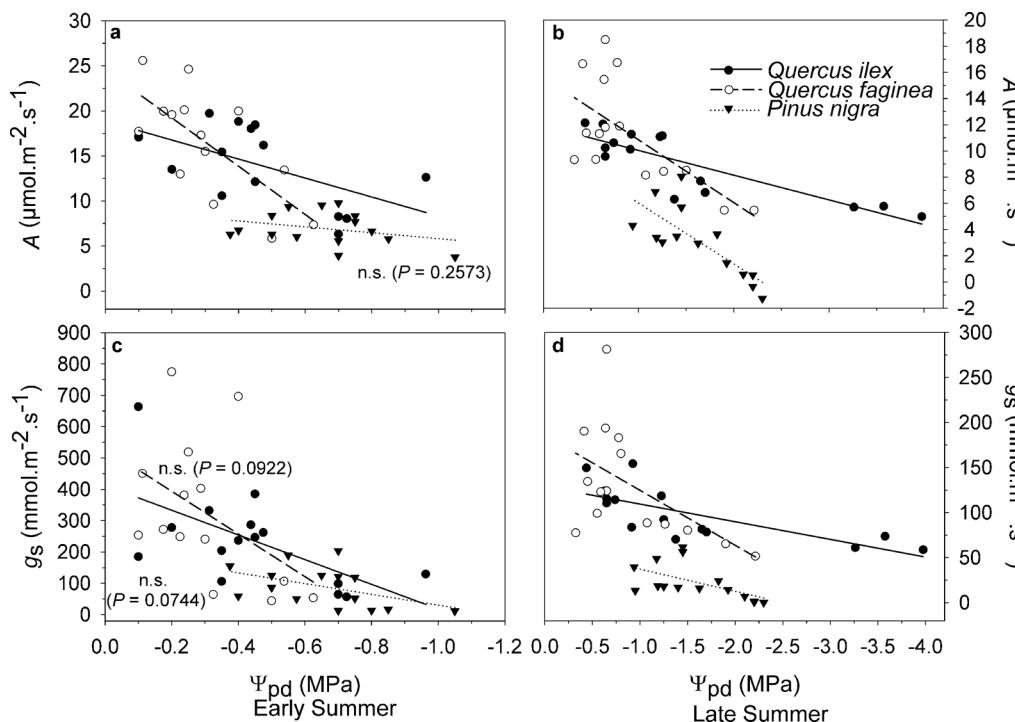
P. nigra showed the highest increase in intrinsic water use efficiency due to increasing dryness. Thus, *P. nigra* had by far the highest plasticity in water use efficiency, which is corroborated by previous studies using other methods as leaf carbon isotopic composition or the ratio between dendrometer and sapflow measurements (Forner et al., 2018). Moreover, this exceptional plasticity in *P. nigra* can be considered an individual strategy to increase further its tolerance to stress (Matesanz

and Valladares, 2014), and maybe it is associated to its water-saving strategy. However, these strategies in *P. nigra* did not compensate the effects of drought and it was translated into severe reductions in carbon uptake already in the early summer in extremely dry years, such as 2012. This highlights the inefficiency of this strategy under severe drought to support continued carbon uptake. In the long term, this strategy could jeopardize the sustainability of *P. nigra*, because of carbon starvation if equally dry periods as in 2012 were more recurrent in the future (Anderegg et al., 2012). However, the period required by species to recover after severe drought should be also evaluated, mainly in response to continuous severe droughts. In fact, *P. nigra* has a high ability to recover growth after droughts in comparison to other coexisting species (Granda et al., 2013), but it could be modified by frequent severe droughts, or even be different in the case of hydraulic conductivity and embolism.

The deciduous *Q. faginea* had higher leaf stomatal conductance and net leaf photosynthetic rate than the evergreen *Q. ilex* during wet conditions and mild droughts, which has been also observed in previous studies (Juárez-López et al., 2008; Mediavilla and Escudero, 2003). These lower rates of carbon gain in *Q. ilex* could be compensated by earlier functional leaves in spring (Gil-Pelegón et al., 2017). However, our results showed that this pattern changed during the severe drought, showing *Q. ilex* had similar or even slightly higher rates than *Q. faginea*. This higher tolerance to severe drought showed by *Q. ilex* was also observed analyzing in detail the whole tree daily stomatal control of water losses. *Q. ilex* kept stomata open at midday even in extremely dry years such as 2012; while *Q. faginea* closed its stomata from early morning onward through the day, avoiding water loss and exhibiting a drought-avoiding strategy throughout the day. Therefore, and within the general drought-tolerant performance of both species, it seems like *Q. ilex* had a wider range of drought tolerance than *Q. faginea* (that showed a tighter control of leaf water loss from early hours in the day (Acherar and Rambal, 1992)). In fact, leaf water relations of both species confirm this claim (see below). The strategy might penalize carbon balance of *Q. faginea*, after integrating from leaf performance to the overall tree, and especially during severe drought conditions. The high coupling between stomatal conductance at the leaf level and sap flow of the tree, together stomatal limitation as the main cause of decrease in leaf net photosynthesis, would give support to the argument of penalizing carbon uptake.

The biochemical characteristics of *Q. faginea* leaves partially compensated its water stress resulting from its stomatal sensitivity to drought, compared with *Q. ilex*. Thus, leaves of *Q. faginea* were more productive as inferred from the highest PNUE of the three studied species, having a lower C_m/N_m , DW/TW, and higher SLA and N_m than *Q. ilex*. These traits are characteristic of highly productive species within the worldwide leaf “economics spectrum” (Wright et al., 2004). Moreover, these traits are linked to higher biochemical capacity combined with higher maximums in stomatal conductance for *Q. faginea* than *Q. ilex* (Juárez-López et al., 2008), but with the exception of severe drought as we have observed. These leaf characteristics would also explain the high leaf PNUE in *Q. faginea*, resulting from the evolutionary trade-off between leaf carbon uptake, leaf lifespan and the construction costs of foliage (Villar and Merino, 2001; Shipley et al., 2006). Even though the drought was very intense, in 2012, it never reached levels that could have affected tree carbon balance of both *Quercus* by altering significantly their phenology by advancing leaf senescence (Montserrat-Martí et al., 2009). On the other hand, *P. nigra* was the species with the lowest PNUE, which was due to being at the opposite end of the range of leaf morphological and biochemical characteristics. Thus, SLA was similar to that of *Q. ilex*, but N_m of pine needles was the lowest of the three species. In this case, leaves with low productivity sum up to the overall higher sensitivity of the stomatal response to drought.

P. nigra had the highest basal water stress during early and late summer, reaching almost -2 MPa of xylem water potential during the



dependent graphs are shown for clarity.

severe drought. This effective water stress endured by *P. nigra* was near to the threshold which separates safety from damage to the hydraulic system at 50% loss of conductivity (-3.2 MPa according to Martínez-Vilalta and Piñol (2003)). In fact, *P. nigra* and *Q. faginea* showed very low K_h during the severe drought. Considering also the insignificant net photosynthesis and the low xylem water potential of *P. nigra* in these conditions, *P. nigra* may have suffered slight embolism. This may explain the huge limitation in gas exchange (Sperry et al., 2002) and its low ability to resist drought (Hacke and Sperry, 2001). Both *Quercus* showed lower K_h than expected during mild drought, which may be an insight of the effects of consecutive extreme droughts. *Quercus* species showed larger drops in predawn water potential than *P. nigra*, but they kept their stomata open through the afternoon. This allowed *Quercus* species to assimilate carbon continuously and to decrease leaf and xylem water potentials to their lowest values during the severe drought.

The 50% loss of conductivity in *Q. faginea* seedlings has been reported to occur at -3.9 MPa (Esteso-Martínez et al., 2006), and in *Q. ilex* adults at -5.6 MPa (Corcuera et al., 2004). Therefore, both *Quercus* species showed a wider range of safety than *P. nigra*, as xylem water potential was far from the above thresholds of xylem dysfunction. This agrees with the idea that drought-tolerant species are less prone to hydraulic failure than drought-avoiding species (Garcia-Forner et al., 2016).

The access to available soil water was species-specific, though the largest drop of Ψ_{pd} for the three species was during the driest conditions. The two oaks seem to have maintained slightly higher values of Ψ_{pd} than the pine in early and late summer. This could be related to a higher capacity to forage for groundwater in both oak species than in the pine (Canadell et al., 1999). This higher capacity to take water from the soil, together with a high maintenance of the hydraulic function

Table 2

Mean \pm SE of the relative water content at the turgor lost point (RWC_{tip} , %), relative apoplastic water content (RWC_a , %), modulus of elasticity (ϵ , MPa), osmotic potential at full turgor (π_o , MPa), osmotic potential at the turgor lost point (π_{tip} , MPa) and ratio of leaf dry weight and leaf turgid weight (DW/TW) at the two sampling seasons, early and late summer (ESu and LSu respectively), for *Quercus* species (Qi: *Quercus ilex*; Qf: *Quercus faginea*) and years (2011–2013). Numbers in bold indicate significant differences between species at $P = 0.05$.

		2011		2012		2013	
		ESu	LSu	ESu	LSu	ESu	LSu
RWC_{tip} (%)	Qi	90.21 \pm 1.24	84.35 \pm 1.47	87.38 \pm 1.61	83.60 \pm 1.15	85.14 \pm 1.33	89.76 \pm 0.51
	Qf	83.92 \pm 1.92	77.83 \pm 1.39	83.75 \pm 1.33	78.64 \pm 2.18	83.48 \pm 1.36	85.56 \pm 1.74
RWC_a (%)	Qi	60.09 \pm 8.48	34.69 \pm 7.01	40.56 \pm 9.53	34.35 \pm 6.33	54.08 \pm 5.99	70.97 \pm 2.19
	Qf	30.38 \pm 3.86	25.33 \pm 7.05	34.72 \pm 6.38	27.71 \pm 6.37	40.85 \pm 5.20	54.39 \pm 4.57
ϵ (MPa)	Qi	7.27 \pm 2.28	11.48 \pm 1.30	11.19 \pm 2.15	12.35 \pm 1.14	6.46 \pm 0.92	6.15 \pm 1.15
	Qf	8.00 \pm 0.69	8.80 \pm 1.01	11.61 \pm 1.43	9.90 \pm 1.44	8.16 \pm 0.72	6.19 \pm 0.69
π_o (MPa)	Qi	-1.75 \pm 0.27	-2.67 \pm 0.13	-2.03 \pm 0.11	-2.96 \pm 0.12	-1.98 \pm 0.16	-1.77 \pm 0.12
	Qf	-1.91 \pm 0.22	-2.37 \pm 0.10	-2.25 \pm 0.16	-2.42 \pm 0.13	-2.06 \pm 0.12	-1.89 \pm 0.15
π_{tip} (MPa)	Qi	-2.54 \pm 0.39	-3.41 \pm 0.10	-2.64 \pm 0.13	-3.91 \pm 0.13	-2.97 \pm 0.23	-2.76 \pm 0.09
	Qf	-2.54 \pm 0.24	-3.31 \pm 0.13	-3.00 \pm 0.18	-3.44 \pm 0.19	-2.87 \pm 0.13	-2.78 \pm 0.12
DW/TW	Qi	0.53 \pm 0.03	0.55 \pm 0.01	0.50 \pm 0.03	0.53 \pm 0.00	0.55 \pm 0.00	0.55 \pm 0.01
	Qf	0.51 \pm 0.03	0.49 \pm 0.01	0.47 \pm 0.01	0.48 \pm 0.00	0.46 \pm 0.01	0.50 \pm 0.01

Fig. 7. Relationship between predawn leaf water potential (Ψ_{pd} , MPa) and both net photosynthetic rate on a leaf area basis (A_{area} , $\mu\text{mol m}^{-2} \text{s}^{-1}$; a, b) and stomatal conductance of water vapor (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$; c, d) measured in the morning for each species and at the two sampling seasons, early (a, c) and late summer (b, d). Significant regressions for early summer: $A_{\text{area}} = 18.87 + 10.55 * \Psi_{pd}$, $R^2 = 0.29$, $P = 0.0215$ and $g_s = 412.25 + 394.28 * \Psi_{pd}$, $R^2 = 0.33$, $P = 0.0143$ (*Quercus ilex*; a and c respectively); $A_{\text{area}} = 24.58 + 25.80 * \Psi_{pd}$, $R^2 = 0.46$, $P = 0.0045$ (*Quercus faginea*; a). Significant regressions for late summer: $A_{\text{area}} = 11.93 + 1.89 * \Psi_{pd}$, $R^2 = 0.70$, $P < 0.0001$ and $g_s = 129.08 + 19.61 * \Psi_{pd}$, $R^2 = 0.52$, $P = 0.0015$ (*Quercus ilex*; b and d respectively); $A_{\text{area}} = 15.63 + 4.79 * \Psi_{pd}$, $R^2 = 0.40$, $P = 0.0066$ and $g_s = 185.78 + 61.04 * \Psi_{pd}$, $R^2 = 0.25$, $P = 0.0337$ (*Quercus faginea*; b and d respectively); $A_{\text{area}} = 10.66 + 4.64 * \Psi_{pd}$, $R^2 = 0.56$, $P = 0.0013$ and $g_s = 61.83 + 24.63 * \Psi_{pd}$, $R^2 = 0.28$, $P = 0.0253$ (*Pinus nigra*; b and d respectively). Note that different scales for independent graphs are shown for clarity.

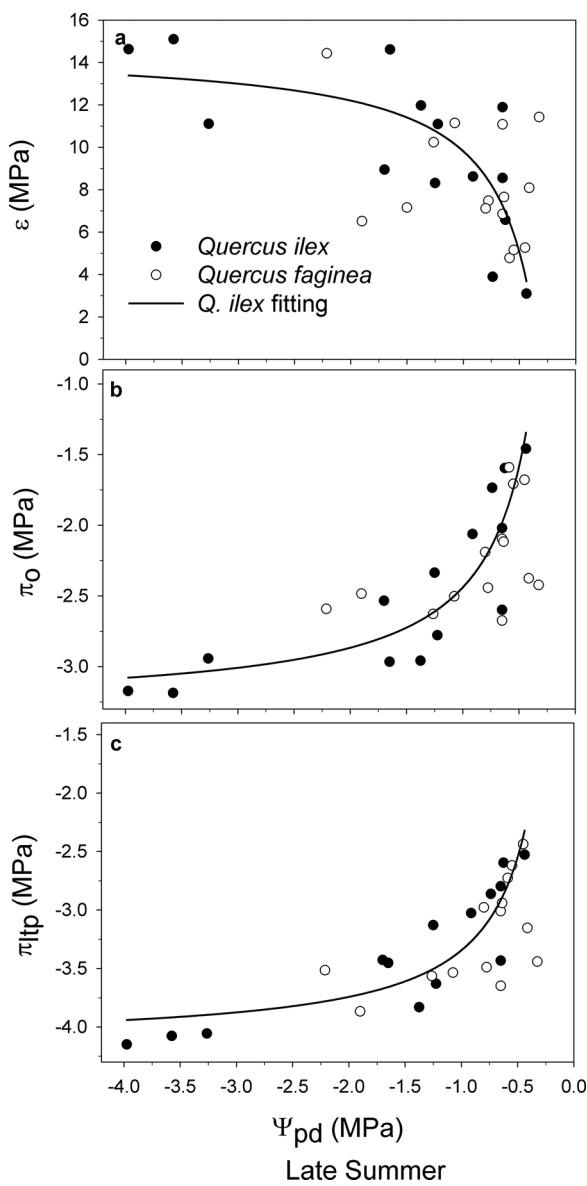


Fig. 8. Relationship between predawn leaf water potential (Ψ_{pd} , MPa) and modulus of elasticity (ϵ , MPa; a), osmotic potential at full turgor (π_o , MPa; b) and osmotic potential at the turgor lost point (π_{tlp} , MPa; c) for *Quercus* species at the late summer season. Significant regressions for *Quercus ilex*: $\epsilon = 14.59 + (4.77/\Psi_{pd})$, $R^2 = 0.56$, $P = 0.0013$; $\pi_o = -3.29 + (-0.85/\Psi_{pd})$, $R^2 = 0.74$, $P < 0.0001$ and $\pi_{tlp} = -4.14 + (-0.80/\Psi_{pd})$, $R^2 = 0.75$, $P < 0.0001$ (a, b and c respectively). No significant regressions for *Quercus faginea*.

especially in very dry years, allowed a higher resilience of both oak species to severe drought. In this respect, functioning of both species in early 2013, regarding most ecophysiological parameters, was in the range of those values recorded in early summer of 2011. However, *P. nigra* recovery was delayed in important parameters such as the K_h evidencing a lower drought tolerance. In addition, drought tolerance of both oaks was reinforced by the leaf osmotic capacity adjustment rendering a decrease in the leaf wilting point stated from π_{tlp} . Minimum values of π_o were observed in the late summer of the driest year, but only in *Q. ilex* the high osmoregulation capacity of the species in response to drought was indicated by the significant relationship between π_o and Ψ_{pd} as a surrogate for the water stress endured by the trees. Relevance of this mechanism and its seasonal and environmental plasticity to tolerate drought has been stated for a long time (Bartlett et al., 2012; Hsiao et al., 1976; Turner and Jones, 1980), and in

particular is important for understanding the ecological consequences of drought tolerance within the genus (Abrams, 1990; Aranda et al., 1996; Castro-Díez and Navarro, 2007; Corcuera et al., 2004). The highest decrease π_{tlp} as consequence of the lowering of π_o would result in higher leaf turgor maintenance in *Q. ilex* than in *Q. faginea*, and the possibility to sustain higher degrees of leaf tissues dehydration as leaf water potentials decreased from early to late summer (Lo Gullo and Salleo, 1988). This combination of traits forms a drought-tolerant performance in the range of water stress experienced, which combined with a high capacity of leaf osmoregulation, would explain the high water use strategy maintained during acute drought periods by the evergreen *Q. ilex* (Nolan et al., 2017).

5. Conclusions

Mediterranean tree species are expected to face longer and harsher stressful periods in the future due to more frequent and extreme droughts (IPCC, 2014). In our study, extreme droughts impacted the water economy of *Pinus nigra* much more, a drought-avoiding species, than the two *Quercus* species, more drought-tolerant species. Water use was drastically reduced by pines with a direct impact on the capacity to uptake carbon. The evergreen *Quercus ilex* maintained a profligate water use at times of maximum water stress due to a combination of mechanisms such as an efficient hydraulic system and the capacity for osmoregulation in leaves that conferred to *Q. ilex* the capacity to maintain leaf turgor under stress conditions. Our results suggest that drought-avoiding species will not avoid severe drought effects and will be at a disadvantage compared with drought-tolerant species, which will still maintain enough margin to tolerate severe drought conditions. Therefore, after several severe droughts drought-avoiding species could show signs of larger decline, with risk of finally dying due to starvation at long term. Hydraulic failure cannot be disregarded as a possible future cause of mortality due to an increase in recurrence of extreme dry periods, but species seems to still have safety margins to cope with this. Despite the high water stress endured by trees, hydraulic function and water use at the tree level recovered well during winter and spring even after the very dry summer of 2012 in both *Quercus* species. Changes in forest composition can be expected due to the differential sensitivity to extremes droughts shown here. This in turn, will potentially affect ecosystem water budgets considering the large differences in water use at the tree level shown by the three species.

Author contribution

A.F. and I.A. got the experimental data. A.F. analyzed the data and wrote a first draft of the manuscript. I.A. and F.V. designed the experiment, provided extensive editing and conceptual advice and reviewed the final version of the manuscript.

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Competing interests

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2018.08.006>.

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